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THE EFFECT OF TREATING THE VIRUS OF TOBACCO MOSAIC WITH THE JUICES OF VARIOUS PLANTS

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During the period that the mosaic disease of tobacco has been under investigation in this laboratory, many attempts have been made to transfer the disease, through cross inoculation, to the pokeweed, *Phytolacca decandra*. Five operators have at different times endeavored to make the transfers referred to, each employing a somewhat different technique. In no instances have results been obtained that might be interpreted as a positive indication of the susceptibility of the pokeweed to the tobacco form of mosaic.

Although at that time no experiment had been made here in the direction of testing the possibility of transfer through insect agency, it seemed quite probable that the pokeweed might be immune. It occurred to the junior author that it might be of interest to determine the effect of the juice of the pokeweed on the tobacco virus. Accordingly, a preliminary experiment was arranged, and it was determined to include in this the effect upon the tobacco mosaic agency of both the juice from healthy pokeweed and that from pokeweed affected with the mosaic peculiar to that host.

Juice or crude sap was then prepared from (1) healthy pokeweed, (2) pokeweed affected with mosaic, and (3) tobacco plants

affected with tobacco mosaic. In each case the procedure was as follows: The younger shoots and leaves were ground up in a meat or food grinder and the resulting semi-fluid mass was thrown into a muslin bag and the juice pressed out. The juice was then filtered through absorbent cotton. The undiluted filtered juice was then used as indicated later. Adequate precautions were taken as to the cleanliness of all vessels involved. The meat grinder and other articles required were, after preliminary cleaning, soaked several hours in hot water containing ½ per cent formaldehyde, and afterwards boiled in plain water. In these and in later experiments where pipettes and other glassware were required, such vessels were invariably dry-sterilized.

In the preliminary experiment, 1 part of the tobacco juice from diseased plants was mixed with 99 parts of each of the 2 types of pokeweed juice, and as a partial control on the dilution and the infectivity of the virus, 1 part of the same diseased tobacco juice was added to 99 parts of sterile distilled water. Further control was not used at the time owing to a scarcity of suitable plants for the inoculation work. The fluids as diluted for inoculation were permitted to stand 3–4 hours prior to their use. The technique of inoculation is described later. Twenty healthy young tobacco plants were inoculated with each of the 3 lots of material, 4 cc. of inoculum being used for each lot. The inoculations were made June 12, and all of the 20 control plants (diseased juice 1 part, water 99 parts) were diseased by June 23, while none of the 40 plants inoculated with the diseased tobacco juice made up in pokeweed juice showed the least indication of mosaic on June 30, when the experiment was closed. These results were so definite that it was determined to repeat the work and to use a variety of plant juices when opportunity offered.

The method of inoculation employed in the preliminary experiments and in that subsequently reported is one that has been adopted as a standard in this laboratory and it requires a brief description. It is preferred to employ young plants with stems about 2 inches high. When possible, the plants to be inoculated are arranged in place about 1 week in advance so that sporadic occurrences of the disease may be promptly detected. Three inoculations are made in each plant, 1 on the stem near the ground,

another farther up and in a leaf axil—so as to reach the young bud—and the other in the immediate region of the terminal bud. The quantity of inoculum required is about 2 cc. for each 10 plants. A large drop of the inoculum may be placed with a glass rod on the spot desired, and with a small inoculating needle many pricks are made through this, thus working the fluid into the tissue. Before passing to the next plant the needle is flamed, then dipped in alcohol, and burned off. The usual precaution is taken with respect to touching plants, or if plants must be touched, the hands are cleansed between operations. Because no disease has appeared in any lot of plants, it is not safe to assume freedom from disease, so that every plant in any lot is treated as though it might convey disease. Inoculated plants are kept under observation for 28–30 days in all cases where more than preliminary data are required.

Preparing the crude sap as already described, experiments were arranged with shoots and leaves of pokeweed, Jimson weed (*Datura Stramonium*), geranium (*Pelargonium* sp.), cotton, and squash, with Irish potato tubers, sweet-potatoes, and apples of the variety Ben Davis. In each case the juice from diseased tobacco plants was diluted, as shown in table I, with the juice from the plant the influence of which was to be tested. In these experiments, the mixed juices were allowed to remain at room temperature about 2 hours, and then placed in a refrigerator, at 3° C., until used the following morning (15–18 hrs.). The undiluted juices used as control were similarly exposed.

From the table it is again clear that pokeweed juice effectively inactivates the agency of tobacco mosaic in a relatively short time. Even when the virus is diluted only 5 times with the pokeweed juice the inhibition is complete. Inactivation is shown by the juice of Jimson weed when the latter is in relatively high concentration, and the juice of the geranium is also to some extent effective. On the other hand, cotton, squash, potato, sweet-potato, and apple exert no injurious influence at the concentrations tested.

It became of much interest to determine if the influence of pokeweed juice especially might result from some relatively simple chemical factor, or if it might be far more complex, possibly

analogous to agglutination. The possibility that the reaction of the juice might be an important factor suggested itself, but colorimetric tests made it clear that the H-ion concentration was

TABLE I

EFFECT OF VARIOUS PLANT JUICES ON THE PATHOGENICITY OF THE TOBACCO MOSAIC VIRUS. TEN PLANTS WERE INOCULATED IN EACH CASE

Juices constituting inoculum	Dilution	Number diseased
Diseased tobacco, dist. water	1 : 10	All (in 15 days)
Diseased tobacco, dist. water	1 : 100	All (in 11 days)
Diseased tobacco, pokeweed	1 : 5	None
Diseased tobacco, pokeweed	1 : 10	None
Diseased tobacco, pokeweed	1 : 25	None
Diseased tobacco, pokeweed	1 : 50	None
Diseased tobacco, pokeweed	1 : 75	None
Diseased tobacco, pokeweed	1 : 100	None
Pokeweed	None	1 (after 4 wks.) (apparently accidental)
Diseased tobacco, Jimson	1 : 10	All (in 9 days)
Diseased tobacco, Jimson	1 : 100	None
Jimson	None	None
Diseased tobacco, Irish potato	1 : 10	All (in 9 days)
Diseased tobacco, Irish potato	1 : 100	All (in 9 days)
Irish potato	None	None
Diseased tobacco, sweet-potato	1 : 10	All (in 11 days)
Diseased tobacco, sweet-potato	1 : 100	9
Sweet potato	None	None
Diseased tobacco, geranium	1 : 10	8 (in 15 days)
Diseased tobacco, geranium	1 : 100	3
Geranium	None	None
Diseased tobacco, apple	1 : 10	All (in 14 days)
Diseased tobacco, apple	1 : 100	8 (in 15 days)
Apple	None	None
Diseased tobacco, tobacco	1 : 10	All (in 12 days)
Diseased tobacco, tobacco	1 : 100	All (in 12 days)
Healthy tobacco	None	None
Diseased tobacco, cotton	1 : 10	All (in 13 days)
Diseased tobacco, cotton	1 : 100	All (in 13 days)
Diseased tobacco, squash	1 : 10	All (in 9 days)
Diseased tobacco, squash	1 : 100	All (in 9 days)
Squash	None	None

higher in the apple juice than in any other tested, and this last exerted no injurious influence.[1] Moreover, experiments then in progress, to be reported elsewhere, indicated a relatively high tolerance of the mosaic virus towards acids. In view of the following facts, namely, that pokeweed is a host for a form of mosaic, that Jimson weed is credited with several forms of mosaic, and

[1] Employing electrometric methods, a subsequent test made by Mr. E. R. Ranker and Miss Fanny Fern Smith gave a P_H of approximately 6.0 for the juice of the pokeweed used.

that even geranium exhibits a disease tentatively classed as a mosaic, the influence of the juices of these plants must be regarded as specific with reference to the tobacco mosaic agency. However, in order to determine if pokeweed juice possesses general germicidal properties, cultures in small Erlenmeyer flasks were arranged with sterile and unsterile (natural) juice, and both lots were inoculated with *Aspergillus niger*. The fungus grew promptly and profusely on both lots, indicating no general inhibiting effect.

The toxicity of pokeweed juice was further tested, using as an indicator the growth of *Bacterium prodigiosum* Lehm. & Neum. In carrying out the experiments with this organism a strong bacterial emulsion was prepared from a fresh nutrient agar slant culture. This emulsion was then added to pokeweed juice,[1] or to an equal quantity of distilled water, as control. These mixtures were allowed to stand for 2 hours, after which suitable dilutions were prepared and plates were poured. Every possible care was taken with the samples employed in the preparation of the dilution cultures to take the sample from the vessel in such manner that the pipette did not come in contact with the walls of the vessel where organisms might occur which had not had free access to the fluid used. In the first series of cultures the original bacterial emulsion was diluted (1) 1:5 with pokeweed juice; (2) 1:25 with pokeweed juice; and (3), for control, 1:25 with distilled water. The results are given in detail in table II.

The result of the foregoing test is convincing proof that no toxicity of pokeweed juice is exhibited toward this species of bacteria. In explanation of the table it should be pointed out that the data with the 2 dilutions of pokeweed juice are closely comparable, while the result from dilution with distilled water shows apparently a very much smaller number of bacteria present. This, however, was anticipated and is in harmony with results obtained from a preliminary series not here reported in detail. This preliminary series brought out the fact that, on

[1] The pokeweed juice used in these experiments was prepared as previously described except that in this case, in order to minimize contaminations, the pokeweed leaves and shoots were first treated for 4 hours with 20 per cent Javel water, after which they were washed in sterile distilled water.

TABLE II

EFFECT OF POKEWEED JUICE ON BACTERIUM PRODIGIOSUM; EXPOSURE TO JUICE 2 HOURS

Treatment	Plate dilution	No. colonies, 4 days	Average, colonies per cc.
Bacterial emulsion diluted 1 : 5 with pokeweed juice	1/10	Numerous	51,930
	1/100	768	
	1/1000	39	
	1/10000	4	
Bacterial emulsion diluted 1 : 25 with pokeweed juice	1/10	960	10,800
	1/100	108	
	1/1000	12	
Bacterial emulsion diluted 1 : 25 with distilled water	1/10	71	1,055
	1/100	14	
	1/1000	0	

standing, an emulsion of this bacterium in distilled water shows an apparent decrease in the number of organisms. It is not the purpose of this paper to determine the cause of this decrease though it may be a simple aggregation phenomenon. For verification of this observation another experiment was carried out, the results of which are briefly given in table III. In this experiment the bacterial emulsion was diluted, as will be seen, with distilled water and with pokeweed juice to the same extent. Plates were poured immediately after the bacterial emulsions were diluted, and then after intervals of 1 and 2 hours respectively.

TABLE III

BACTERIUM PRODIGIOSUM IN DISTILLED WATER AND IN POKEWEED JUICE

Treatment	No. colonies, initial	No. colonies, after exposure of 1 hour	No. colonies, after exposure of 2 hours
Bacterial emulsion diluted 1 : 25, distilled water	20,300	—	7,050
Bacterial emulsion diluted 1 : 25, pokeweed juice	29,850	44,050	42,500

No discussion of table III is required further than to point out again the diminution, on standing, of the number of colonies in the case of the emulsion diluted with distilled water. The figures for the emulsion diluted with pokeweed juice are sufficiently comparable when it is recalled that this is not filtered juice, and

accordingly represents a minute suspension, in itself a sufficient cause for some variation in numbers.

These experiments were further suggestive of a specific inactivating effect of the pokeweed juice towards the agency of mosaic disease. The cases of geranium and of Jimson weed have not been more closely analyzed.

It seemed desirable to determine the possible relation of the larger colloidal particles in the pokeweed juice to inactivation, but up to the present only a few preliminary experiments have been made. These, however, are not without suggestion. Pokeweed juice was filtered through a cylindrical, porcelain atmometer cup under a pressure of one-half atmosphere, and the filtrate thus obtained was used in treating the mosaic virus subsequently used in inoculations, as in table I. When the relation of virus to filtrate was 1 : 10, the incidence of infection was 9 out of 10 plants inoculated; when the relation was 1 : 100, the virus was completely inactivated. Filtration through such a filter seems therefore to reduce the effect of the juice. It may be stated that the filter employed permits the mosaic virus to pass through but does not permit *Bacterium prodigiosum*.

Some of the same lot of pokeweed juice was diluted with an equal quantity of distilled water and then centrifuged for 30 minutes at 1500 revolutions per minute. The effect of the supernatant liquid on the virus was determined, as before, by inoculating 10 tobacco plants. With the relation of the diseased juice to this diluted liquid 1:10, 2 plants developed the disease, again indicating some loss of inactivation capacity.

It seemed conceivable that inactivation of the mosaic virus might be due to adsorption. It was not possible to determine this merely by increasing relatively the quantity of diseased tobacco juice, since this dilutes the pokeweed juice. Concentration of the diseased tobacco juice by evaporation would also be subject to criticism from another angle. Further work on the chemical or physical nature of this inactivation is planned.

For the moment the data in the 3 experiments given in table IV show further the influence of the ratio of diseased juice to pokeweed juice as developed through infection experiments, and thus supplement table I. The experiments were made under con-

ditions similar to those reported in table 1, but at a different time. The progressive increase in the incidence of disease with the increasing amount of virus locates rather definitely, in conjunction with the data of table 1, the inactivation capacity of the pokeweed juice.

TABLE IV

Juices constituting inoculum	Dilution	Result
Diseased tobacco, pokeweed	1 : 1	2 diseased (in 8 days)
Diseased tobacco, pokeweed	5 : 1	8 diseased (in 14 days)
Diseased tobacco, pokeweed	10 : 1	9 diseased (in 9 days)

Upon the completion of the experiments with pokeweed juice reported in table 1, the results were regarded, and apparently properly so, as a simple case of inactivation. Nevertheless, it was considered desirable to ascertain if in the plants used in experiments 3–8 inclusive there might exist such a mild form of the disease as to be essentially without external symptoms. Accordingly, leaves of these plants were used directly in the inoculation of fresh young tobacco plants, but without visible result. Likewise, one-half (5) of each lot of plants used in experiments 3–8, table 1, were inoculated with diseased tobacco juice, untreated, and the entire lot of 30 plants exhibited the characteristic mosaic symptoms in less than 15 days, indicating, as anticipated, not the least suggestion of a modification of susceptibility to mosaic.

The specific nature of this inactivation of the tobacco mosaic virus, especially by pokeweed juice, has seemed of sufficient importance to justify the projection of further experiments in the direction of ascertaining the physical and chemical properties of this juice.